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14. ABSTRACT

Stromal-epithelial interactions regulate breast cell fate via integrin-growth factor receptor interactions that activate tyrosine kinases that are tempered by protein tyrosine phosphatases (PTPP). Through various screening approaches we identified and profiled PTP expression in normal, malignantly transformed and phenotypically-reverted breast tissue and identified the Band 4.1 PTPs MEG1 and D1 as candidate PTP metastasis suppressor genes. We demonstrated that MEG1 and D1 expression rise dramatically early during mammary morphogenesis in response to cues from a compliant laminin-rich basement membrane that inhibit focal adhesion maturation and promote adherens junction assembly. The expression of PTP MEG1 and D1 is thereafter rapidly down regulated coincident with polarity and growth arrest. We also found that MEG1 and D1 levels are altered in tumors and that a stiffer tumor-associated stroma disrupts tissue organization and MEG and D1 expression and promotes malignant behavior of MECs. Because inhibiting mechanical force could restore tissue organization and normalize MEG and D1 expression, we are currently exploring the possibility that a reactive "stiffer" ECM stroma might drive malignant transformation of the breast by altering PTP function to disrupt tissue organization.

15. SUBJECT TERMS
protein tyrosine phosphatase, tyrosine kinase, PTP-Meg 1, PTP-D1, Band 4.1 PTP, integrin, architecture, adherens junction, ECM, 3D

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INTRODUCTION:

Stromal-epithelial interactions and integrins regulate mammary gland development and homeostasis, and modulate tumor progression and treatment responsiveness (Unger and Weaver, 2003; Zahir and Weaver, 2004). Although it is not known how the stroma exerts such a profound effect on mammary epithelial cell (MEC) function, studies in culture have demonstrated that the basement membrane (BM) extracellular matrix (ECM); the insoluble protein component of the stroma surrounding MECs in vivo) regulates MEC growth, survival, migration and differentiation through transmembrane receptors called integrins (Miranti and Brugge, 2002). Integrins mediate their biological effects by initiating biochemical and biophysical signaling that involves protein tyrosine phosphorylation events and that also rely upon cooperative interactions with growth factor receptors and cytoskeletal interactor/modifier molecules (Moghal, 1999, Assoian and Schwartz 2002). The level of tyrosine phosphorylation induced by integrin and growth factor receptor activation is dynamically regulated by the concerted action of protein tyrosine kinases and protein tyrosine phosphatases (PTPs). Several lines of evidence suggest that both transmembrane and intracellular PTPs play an important role in mammary morphogenesis (Aoki, 1999, Schaapveld, 1997). Indeed, culture studies have implicated PTPs as critical regulators of cell-cell interactions (Aberle, 1996), integrin-mediated signaling (Angers-Loustau, 1999, Angers-Loustau, 1999), and focal adhesion assembly (Vadlamudi, 2002), and as modifiers of integrin-growth factor receptor interactions (Moghal, 1999, Angers-Loustau, 1999). However, very little is known about the molecular functions and substrate targets of PTPs in adhesion-dependent MEC behavior. Thus the overall goal of our studies is to delineate the importance of and identify plausible mechanisms whereby PTPs regulate, and are themselves regulated by ECM-integrin-growth factor receptor signaling, and thereafter to determine the role of PTPs in breast cancer progression.

To achieve our goal we have been studying normal and perturbed morphogenesis of MECs using a human breast cancer model called HMT-3522, which progresses from non-malignant S-1 through pre-malignant S-2 to tumorigenic T4-2 MECs (Rizki and Weaver et al., In Revision), as well as using a non-malignant immortalized MEC line established from a fibrocystic breast lesion called MCF10A (Muthaswamy et al., 2001). When used in conjunction with a reconstituted BM assay these MECs recapitulate the morphological and behavior of MECs as they transit from a non-transformed, through to pre-malignant and finally to malignant, invasion breast tissue phenotypes. Using these MEC lines our studies have clearly established that the progressive malignant behavior of the cells in this series is linked to alterations in integrin-growth factor expression and activity and perturbed MEC-ECM responsiveness because we are able to phenotypically revert our mammary tumor cells in culture, and in vivo by inhibiting the activity of either β1 integrin and/or the epidermal growth factor receptor (Weaver et al., 1997; reviewed in Unger and Weaver, 2003). Alternately, we can drive the malignant behavior of non-malignant and the pre-malignant MECs by altering the expression of specific integrin heterodimers such as α5β1 and α6β4 integrin (Zahir et al., 2003; Rozenberg et al., Submitted; Friedland et al., Submitted, unpublished observations).

We successfully identified 15 PTPs whose mRNA levels were regulated during adhesion-directed morphogenesis in MECs and that were modified in transformed MECs. Specifically, we noted that the Band 4.1 PTPs PTP Meg1, D1, H1 and FAP were decreased in differentiated 3D mammary tissues and that their expression was elevated in transformed cells. Data showed that PTP Meg 1 and D1 were significantly increased 24 hours following the interaction of nonmalignant MECs with a reconstituted BM, and prior to differentiation whereas the tumors did not appropriately modulate PTP expression in response to ECM cues. Studies have implicated MEG1 and D1 in the regulation of cell adhesion, formation of focal adhesion and cytoskeletal organization, and their dysregulation has been implicated in uncontrolled proliferation and altered cell-ECM interactions (Gutmann, 2001; Ogata, 1999, Ogata, 2999, Wadham, 2000) and we and others showed that they are robustly regulated during

normal mammary morphogenesis in the developing mammary gland in vivo. Accordingly, our working hypothesis is that: **PTP Meg1 and D1 play pivotal roles in ECM directed MEC morphogenesis and that their dysregulation contributes to malignant transformation and acquisition of an invasive, metastatic breast tumor phenotype.**

To test this hypothesis our studies have been designed to complete the following specific aims:

Specific Aim 1: Generate PTP-Meg 1 and D1 antibodies and then characterize the expression, activity and localization of these endogenous PTPs in normal and dyregulated BM-directed MEC morphogenesis, and identify the morphological parameters linked to their regulation.

Specific Aim 2: Analyze the cellular and molecular consequences of over expression of PTP-Meg 1 and D1 wild type and dominant negative mutants during tumor progression and following phenotypic reversion.

Specific Aim 3: Identify and characterize known and/or novel PTP-Meg 1 and D1 substrates/binding porteins and/or cellular targets that may lie on pathways implicated in Specific Aims 1 and 2.

Summary of Achievements - Proposal Body:

To characterize the expression, activity and localization of PTP-Meg1 and D1 in normal and deregulated rBM-directed MEC morphogenesis, and to identify the morphological parameters linked to their normal and abnormal regulation.

Survey of PTPs expressed in human breast tissue

		Identification Method		Verification Method		
PTP (Locuslink)	Synonyms (From Locuslink)	RFDD	Cloning	PCR/RT-PCR	RNase Protection	Immuno-blot
PTPN12	PTP-PEST, PTPG1	Y	Y	Y	Y	Y
PTPN13	FAP1, PTP1E, PTP-BAS, PTPL1	Y	Y	Y	Y	
PTPN21	PTPD1, PTPRL10	Y	Y	Y		
PTPN1	PTP1B, PTP-1B	Y		Y		
PTPN6	HCP, HCPH, SHP-1, PTP-1C, SHP-1L	Y		Y	Y	Y
PTPN9	PTP-MEG2	Y	Y	Y	Y	
PTPN4	PTP-MEG1	Y	Y	Y		
PTPN2	PTPT, Tcell PTP					
PTPRU	PI, FMI, PTP, PCP-2, PTP-J, PTPRO, PTPU2, GLEPP1, HPTP-J, PTP-PI, R-PTP-PSI	Y		Y	Y	
PTPRA	LRP, HLPR, PTPA, HPTPA, RPTPA, PTPRL2		Y	Y		
PTPRK	R-PTP-	Y	Y	Y	Y	Y
PTPRG	PTPG, HPTPG, RPTPG, D3S1249	Y	Y	Y		
PTPRS				Y		
PTPRF	LAR			Y		
PTPRJ	DEP1			Y		

Table 1. Through microarray analysis, restriction fragment differential display, and cloning techniques, we identified 15 PTPs expressed in MECs. This data was verified through PCR/RT-PCR, RNase protection assays, and immunoblots.

One of our first objectives has been to methodically characterize changes in Band 4.1 PTPs during normal morphogenesis and following malignant transformation in the breast and thereafter to assess the state of PTPs in reverted tumor tissues. Towards this goal we have conducted a comprehensive analysis of PTP expression in normal, tumorigenic and reverted tumors using RT-PCR, immunoblotting, immunostaining and RNAse protection analysis to assess the role of PTP Meg1 and D1 in MECs in both 2D monolayer conventional cell culture and in a 3D reconstituted basement membrane (rBM)-directed tissue morphology format. The results of these studies were discussed at length in an earlier report so that here we will simply summarize these results and highlight our newer observations. To begin with we have constructed a comprehensive profile of PTPs expressed in normal human breast tissue (see Table 1; see also appendix 1). In earlier reports we showed data primarily obtained concerning the state of PTPMeg1 and D1 in nontransformed and transformed MECs and in 2D and 3D culture formats. These studies clearly showed that PTPMEG1 and D1 expression is significantly higher in non-differentiated MECs grown in a 2D culture format (see earlier report) – and they show that MEG1 (principally) and to a limited extent D1 are transiently up-regulated in nonmalignant MECs embedded within rBM. This transient expression occurs rapidly and is followed by a quick and dramatic reduction in mRNA levels such that PTPMEG1 and D1 expression are down regulated and barely detected in differentiated, polarized, growth-arrested mammary acini by 10 days of culture (see Figures earlier report). Importantly, we conducted time course studies of mammary growth and colony size and could show that PTPMEG1 and D1 transient up regulation occurred prior to induction of MEC proliferation and that the expression of these PTPs was decreased several days prior to growth-arrest of the spheroids (see Figures earlier report).

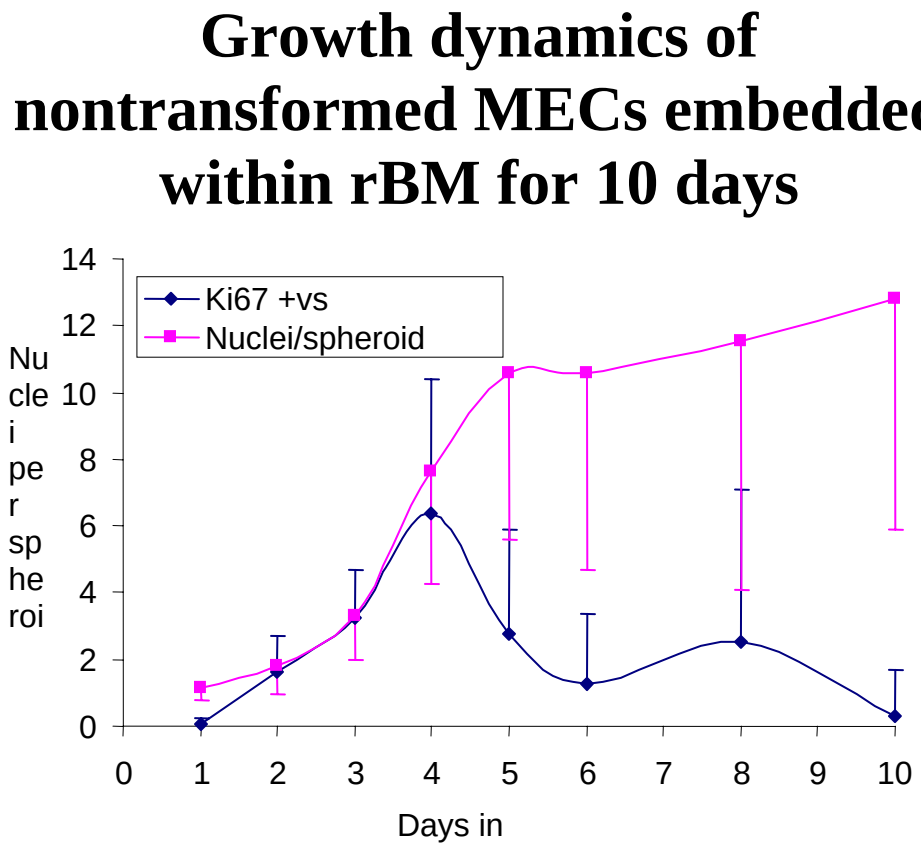

Figure 1. Human MECs embedded within rBM as single cells begin to proliferate rapidly within 24 hours and continue cycling until they polarize at day 4, thereafter they exit the cell cycle and growth arrest between 6 and 8 days (dark blue line). Consistently, colony size increases dramatically until 5-6 days and thereafter remains constant.

PTPMEG1 and D1 are differentially regulated in 2D and 3D and between nonmalignant and malignantly transformed MECs

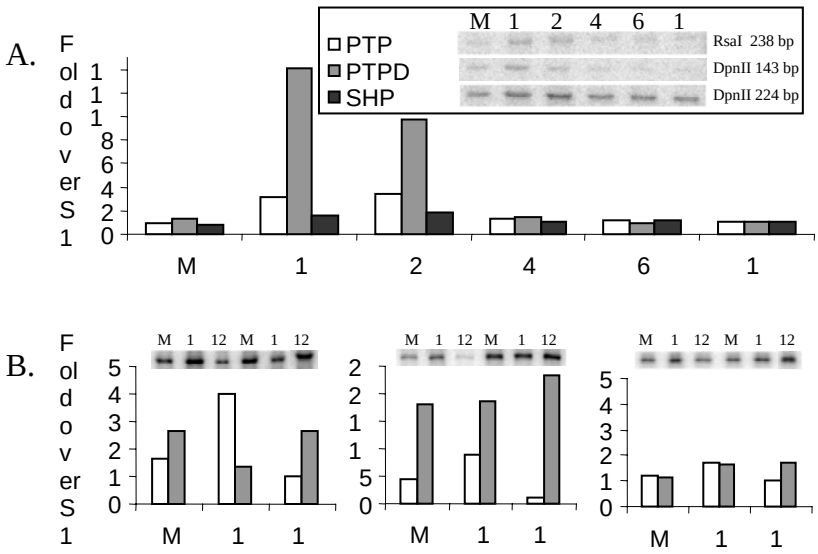

Figure 2. Tyrosine phosphatases are expressed at higher levels in normal S-1 MECs early during morphogenesis in rBM, decreasing to low levels by day 4 to day 12. Expression is higher in tumorigenic T4-2 MECs that do not undergo morphogenesis and PTP expression fails to down regulate.
A.) Restriction fragment differential display (RFDD) of degenerate RT-PCR of "classical" PTPs in normal human mammary epithelial cells (S-1 MECs) grown as monolayers or embedded for the indicated number of days in rBM.
B.) Normal MECs (S-1) expression levels are compared to tumorigenic MECs (T4-2) . Unique RFDD bands in parentheses give restriction endonuclease, 5' or 3' end label, and predicted size that correspond to the indicated PTPs.

NOTE: We have profiled PTPs in additional nontransformed MECs and transformed cell lines to ensure that the results we have obtained using the HMT-3522 cell series is representative of the state of PTPs in all human breast systems. To achieve these results we prepared RNA from several time course experiments. We then profiled and quantified the expression of PTPs using RT-PCR from MECs on standard monolayers between days 1 and 10 on tissue culture plastic and on a thin layer of laminin-rich rBM and those grown within rBM between 24 hours and 16 days. This was done in order to assess the effect of cell-cell interactions and growth status in addition to a 3D ECM format on PTP expression in MECs. In parallel we assayed MECs for growth status using BrDU, Ki-67 and PCNA status and by cell counting and colony size measurements to determine if changes in expression could be linked to altered growth status. We also conducted immunostaining for indicators of adherens junction assembly (beta catenin and E cadherin), tissue polarity (basal localization of $\alpha 6\beta 4$ integrin and apically organized F-actin) and deposition of an endogenous, organized basement membrane (basally-deposited laminin 5 and collagen IV)(these results were shown in a previous report). Primer results were quantified as per standard methods and three-five independent experiments were analyzed. MECs embedded within a rBM as single cells begin to proliferate within 24 hours and continue to cycle rapidly for 4 days and then rapidly growth-arrest and exit the cell cycle within 8 days as illustrated by cells that were positive for Ki-67. Consistent with these changes in growth status, colony size increased rapidly and progressively until 8 days and thereafter the colony size remained constant (see Figure 1). Morphological analysis

showed that within 48 hours cells embedded within a rBM had doubled and thereafter assembled cell-cell adherens junctions, and that by 4 days the structures consisting of 4-6 cells were polarized, but that assembly of an endogenous basement membrane did not occur until the cells had fully growth-arrested and exited the cell cycle which occurred by days 8-10 (see previous progress report for images). Data obtained from RNA collected at similar times demonstrated that Band 4.1 PTP Meg 1 and D1 are significantly higher in nontransformed MECs grown on a 2D monolayer as compared to within rBM. However nonmalignant MECs exhibit a transient burst of MEG1 and D1 expression coincident with adherens junction assembly and irrespective of growth status. These data suggest that MEG1 and D1 expression are NOT coupled to pathways linked to regulation of MEC growth. Instead our earlier studies indicated that PTPMEG1 and D1 down regulation were associated with assembly of cell-cell adherens junctions (refer to earlier Figures presented in previous Progress report). We have also conducted time course studies to assess the levels of expressed MEG1 and D1 in malignantly transformed MECs in rBM. We found that levels of PTPMEG1 and to a lesser extent D1 remained similar to what we had initially detected in the tumor cells on tissue culture plastic (see Figure 2). Furthermore, we could not detect any transient up regulation of MEG1 or D1. We had conducted preliminary studies to assess the effect of phenotypic reversion of the tumors to determine if MEG1 and/or D1 expression might play a critical role in driving tumor dormancy. Here we report the results of additional and completed analysis of the state of PTP expression in the transformed and phenotypically reverted mammary tumors. Our data clearly demonstrate that phenotypic reversion inhibits the expression of most of the PTPs we surveyed including PTPD1 (Figure 2). However, and surprisingly, although initial studies had suggested that PTPMEG1 expression was NOT changed by phenotypic reversion – we have now repeatedly confirmed that PTPMEG1 expression levels become dramatically up regulated in mammary tissue-like structures following phenotypic reversion (see Figure 3).

Phenotypic reversion and normalization of tumor tissue represses the expression of the majority of PTPs including PTPD1 however PTPMEG1 becomes substantially elevated suggesting PTPMEG1 is necessary for maintenance of tumor dormancy

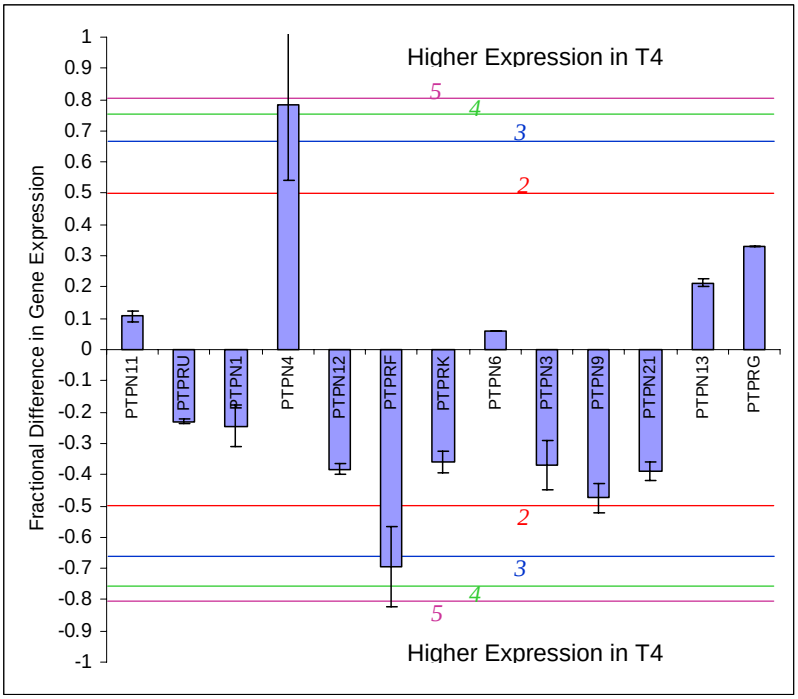

Figure 3. RT-PCR and Q-PCR results summarizing results showing that phenotypic reversion of tumors either by inhibiting epidermal growth factor signaling, beta 1 integrin adhesion-dependent signaling OR Rho-ROCK-myosin contractility NORMALIZES the expression of the majority of PTPs expressed in MECs. However, PTPMEG1 expression becomes greatly induced such that its expression becomes almost 5 fold greater than that expressed in tumor cells – similar to the elevated expression transiently expressed in NORMAL MECs during mammary morphogenesis and coincident with assembly of cell-cell adherens junctions. These data indicate that PTPMEG1 may play a critical role in inducing tumor dormancy by regulating cell-cell junctional integrity.

Interestingly, immunoblot data indicate that PTPMEG1 levels decline somewhat in the reverted tumors as compared to the tumors. Because our earlier studies clearly indicated a close relationship between RNA and protein expression of PTPs we have initiated studies to explore whether PTPMEG1 has an additional level of feedback regulation at the protein level not previously revealed. Indeed, ectopic expression of PTPMEG1 has been exceedingly difficult to achieve – such that despite high levels of RNA expression we often are only able to obtain two to three fold increases at the protein level (ongoing experiments that are to be included in next years report). This again suggests that PTPMEG1 may be controlled at both the RNA and the protein levels. Collectively these data suggest that Meg 1 and/or D1 are likely functionally upstream of pathways that are critical for formation of a differentiated tissue structure, such as those involved in driving the assembly of stable adherens junctions, although we have yet to rule out the possibility that formation of a stable tissue structure might itself alter PTP Meg 1 expression. The relevance of these observations will need to be clarified in the next two years and should be greatly facilitated following assessment of changes in PTP activity and subcellular localization in differentiated, tumor and phenotypically reverted MEC tissues, as well as following the identification of PTP Meg 1 and D1 cellular substrate targets.

Establishment of Functional links between matrix stiffness, integrins and PTPs in the regulation of MEC morphogenesis

Similar ECM stiffness between rBM (Matrigel) 3D substrates and Mammary gland and elevated stiffness of conventional 2D monolayer tissue culture subtrata (plastic and borosilicate glass)

Tissue or Material	Elastic Modulus (Pa)
Mouse Mammary Gland	167 ± 14
Matrigel [a]	175 ± 37
Collagen (2.0 mg/ml) [b]	328 ± 87
Collagen (4.0 mg/ml) [b]	1589 ± 380
Plastic (polystyrene) [c]	2.78×10^9
Glass (soda-lime) [c]	69×10^9

TABLE 2. The ECM stiffness of the mammary gland in vivo and rBM substrates such as Matrigel or collagen I substrates are remarkably similar with respect to their viscoelastic properties. In contrast the stiffness of standard tissue culture plastic (polystyrene) and glass (borosilicate or soda lime) is significantly stiffer by several orders of magnitude.

Thus far all of our experiments have concentrated towards defining the changes in PTPMEG1 and D1 in nonmalignant, transformed and phenotypically reverted tumors in rBM. Towards understanding the molecular relevance of PTP dysfunction in tumors and its putative role in mammary morphogenesis we have begun to explore the molecular basis of adhesion-dependent tissue differentiation. Our studies have clearly demonstrated that one major regulator of epithelial differentiation is the physical or material properties of the surrounding stromal extracellular matrix (ECM). Thus while it has been greatly appreciated that the ECM stroma can profoundly regulate epithelial behavior by engaging integrins and other transmembrane receptors the effect of physical presentation of the matrix has been essentially ignored. Here we report (and have recently published) that ONLY physical conditions that are identical in their visco elastic properties or stiffness to that found in the mammary gland in vivo are able to support mammary epithelial morphogenesis. Thus we measured the stiffness (visco elastic properties herein quantified in Pascals) of a NORMAL mammary gland in vivo. We could show that the mammary gland in a normal mouse (similar to what we have now determined in a human mammary gland) is highly compliant (soft). We also noted that rBM or Matrigel which promotes mammary morphogenesis has a similar soft consistency as do low concentrations of collagen. In marked contrast however, tissue culture plastic substrates such as polystyrene and borosilicate glass are significantly and dramatically stiffer (see Table 2). To directly test this phenomenon we manipulated the stiffness of the matrix by either increasing the collagen concentration, collagen cross linking or through the use of laminin-ligated or rBM-cross linked polyacrylamide gels and examined the effects of progressive stiffening of the ECM on mammary morphogenesis. As can be seen from Figure 4, as the matrix is progressively stiffened tissue morphogenesis becomes substantially compromised. Initial effects include a failure to clear the lumen (a hallmark of DCIS lesions) followed by a destabilization of cell-cell junctions and finally a loss of basal and apical polarity. The consequence of perturbing epithelial morphogenesis is that MECs encountering a highly stiff matrix form continuously growing, abnormal colonies reminiscent of premalignant mammary lesions (Paszek et al., 2005).

Although the effect of matrix stiffness on MEC morphogenesis is striking and intriguing – with out a clear indication of molecular mechanisms definitive conclusions regarding links to PTPs and tumors is not clear. As such we undertook an exhaustive analysis of the effect of matrix stiffness on integrin and growth factor receptor signaling. Much of these results have been published in our Cancer Cell article Paszek et al., 2005 Cancer Cell and have also been discussed in a review article published in 2005 in the Journal of Mammary Gland Biology and Neoplasia Paszek and Weaver. Here we summarize our major finding which is that matrix force compromises mammary morphogenesis primarily by CHANGING the nature of the integrin adhesion structure formed (see Figure 5). Thus matrix force drives the assembly of 397FAK, and vinculin containing focal adhesions that then lead to the recruitment of multiple signaling molecules that enhance signaling through pathways linked to integrins such as PI3 kinase and ERK. Interestingly, in the course of our studies we could show that mammary tumors IN VIVO are significantly stiffer than normal tissue and that the mammary stroma (surround ECM) stiffens significantly in vivo prior to tumor formation. Consistently, MECs residing within a stiff matrix in vivo have assembled mature focal adhesions containing 397FAK and vinculin (see Figure 6).

Encouraged by our striking observation that tissue stiffness was associated with an abnormal tissue behavior in the breast and that tissues stiffen appreciably prior to tumor formation – we examined the contractility or force-generating behavior of breast tumor cells. We found that breast tumors exert considerable more force towards their matrix than nonmalignant MECs and more dramatically that we could normalize the behavior of these tumors by inhibiting their integrin-dependent, force-linked signaling pathways –integrin/growth factor receptor- Rho-ROCK-myosin. These observations may have considerable relevance to understanding and treating breast tumors (see Paszek et al., 2005 and Paszek and Weaver 2004).

Extracellular Matrix stiffness perturbs cell-cell adherens junctions, disrupts polarity and compromises mammary morphogenesis

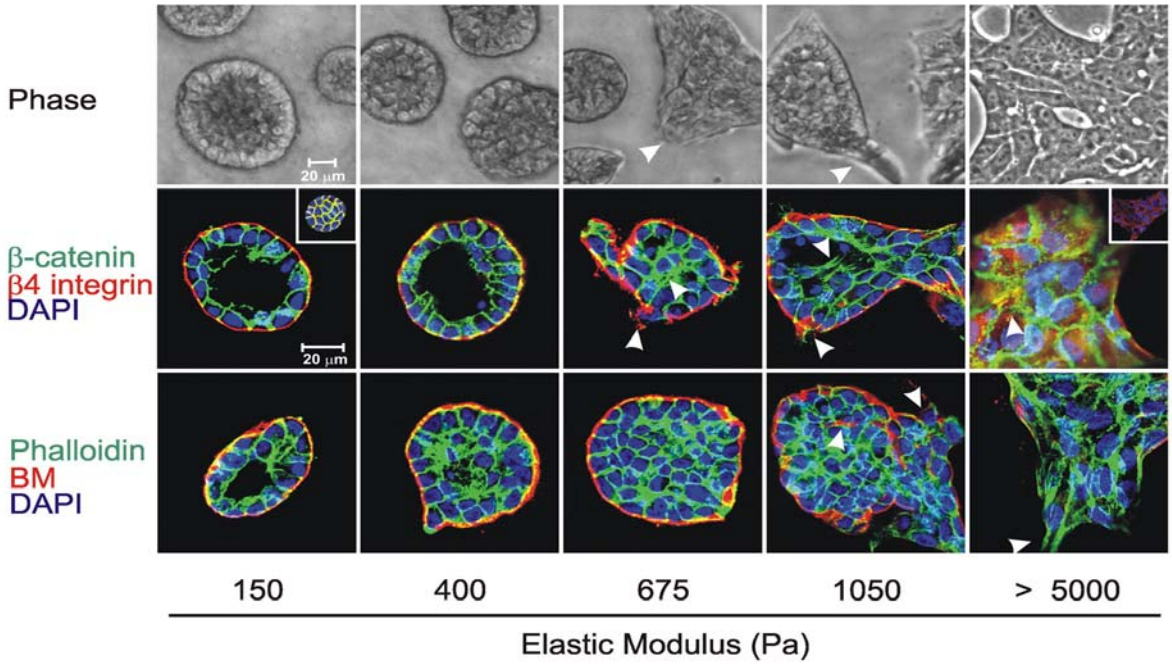

Figure 4. Phase contrast microscopy and confocal immunofluorescent images of MEC colonies on 3D rBM-crosslinked polyacrylamide gels of increasing stiffness (150–5000 Pa), showing colony morphology after 20 days (top). β-catenin before (large image) and after triton extraction (inset), costained with β4 integrin (large image) or E-cadherin (inset).

Increased substrate stiffness induces focal adhesion maturation in culture and in vivo

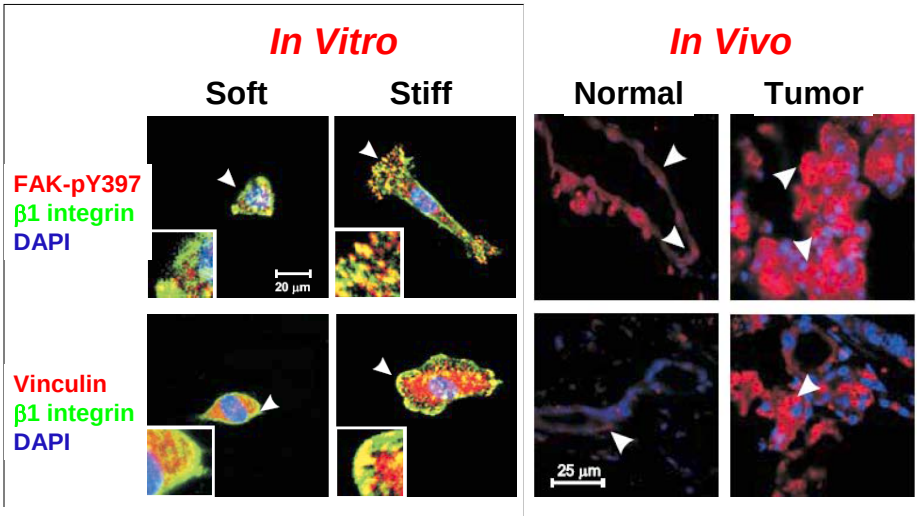

Figure 5.

. MECs interacting with highly compliant matrices form small immature focal contacts (b1 integrin; green) lacking 397FAK (top panel red) and vinculin (red; bottom panel) whereas they assemble mature focal adhesions with 397FAK and vinculin in response to a stiff matrix. Similarly, MECs incorporated into a "normal" tissue in vivo encounter a highly compliant (soft) matrix and lack 397FAK (red; top; right panel) and vinculin (red; bottom; right panel) whereas MECs within transformed mammary tissues which are highly stiff have abundant 397FAK (far right top; red) and vinculin (far right bottom; red).

More relevant to the thesis of this proposal, considering the potential importance of force to normal and tumor behavior in the breast we assessed the effect of matrix force on PTP expression and specifically illustrate here effects of matrix force on PTPMEG1 expression. Strikingly, we could consistently demonstrate that nonmalignant MECs interacting with a highly stiff matrix exhibited greatly elevated expression levels of PTPMEG1 mRNA (see Figure 6). We are now exploring the importance of this observation to mammary morphogenesis. In addition, given that protein and mRNA expression of PTPMEG1 may not be directly linked we are also assessing the effect of matrix force on PTPMEG1 expression at the protein level.

Matrix stiffness induces PTP-MEG1 expression – suggesting that a functional link exists between adhesion-dependent force and PTP MEG1

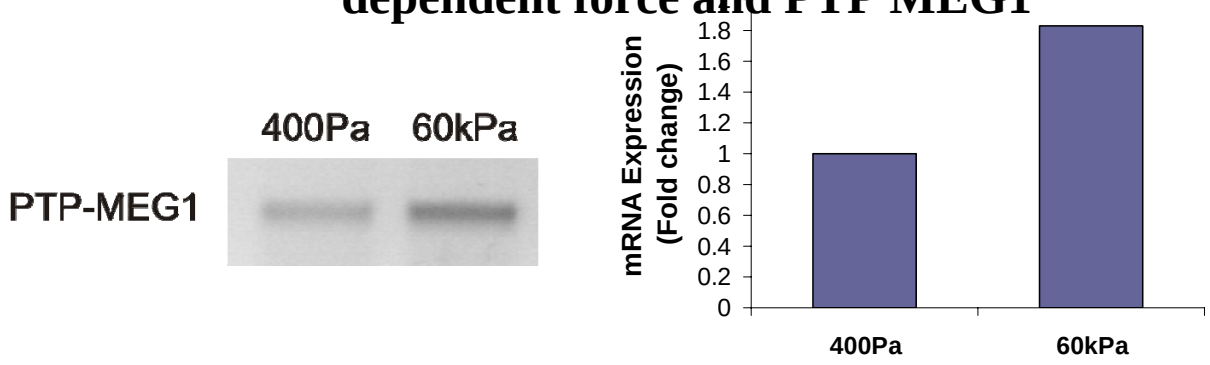

Figure 6. Semi-quantitative RT-PCR of MEC colonies on soft (400Pa) and stiff (60kPa) rBM-crosslinked polyacrylamide gels showing that mRNA expression on PTP-MEG1 is increased in MECs plated on a stiff substrate.

KEY RESEARCH ACCOMPLISHMENTS:

- Completed Semi-quantitative RT-PCR and Q-PCR analysis of PTP expression during MEC morphogenesis and comparison to 2D monolayer conditions
- Continuation of assessment of protein changes during MEC morphogenesis and comparison to 2D monolayers using the procured polyclonal antibody to PTPMEG1 via immunoblot analysis
- Preliminary immunostaining studies conducted
- Completion of studies assessing functional links between PTPMEG1 (N4) and cell proliferation and cell cycle control
- Completion of studies assessing functional links between PTPMEG1 (N4) and cell-cell adherens junctions
- Completion of studies assessing functional links between PTPMEG1 (N4) and tissue polarity in nonmalignant HMT-3522 AND MCF10A MECs in both 2D and 3D culture formats
- Completion of RT-PCR and Q-PCR analysis of PTP Meg1 (N4) expression in normal, tumor and phenotypically reverted rBM-directed 3D tissue-like structures
- Completion of immunoblot analysis of protein status of PTPMEG1 (N4) in nontransformed, tumors and phenotypically-reverted MEC tissue-like structures
- Identified an integrin-linked and growth factor linked regulatory pathway that influences PTPMEG1 expression
- Completed the characterization of integrin-linked/force dependent regulation of mammary morphogenesis
- Completed the characterization of effect of exogenous and endogenous force on mammary morphogenesis
- Preliminary studies characterizing the effect of exogenous matrix force (stiffness) on PTPMEG1 expression
- Completed the generation of an array of tagged, untagged and bicistronic PTPMEG1 (N4) expression constructs for retroviral inducible/repressible expression as well as adenoviral and lentiviral expression generated.

Reportable Outcomes:

A. Manuscripts

1. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, Hammer DA, **Weaver VM.** Tensional homeostasis and the malignant phenotype. Cancer Cell 2005; 8(3): 241-254.

2. Paszek, M. and **Weaver V.M.** The tension mounts: mechanics meets morphogenesis and malignancy. J Mammary Gland Biol Neoplasia. 9:325-42, 2004.

B. Abstracts

1. Zahir N, Johnson KR, Lakins JN, Paszek M, Gasser D, Margulies SS, **Weaver VM.** Spatial-Mechanical Regulation of Mammary Epithelial Cell Morphogenesis. Poster Presentation. BMES Annual Fall Meeting, Philadelphia, PA, October 2004.

2. Paszek, M., Zahir, N., Lakins, J.N., Lynch, L., Boettiger, D., Hammer, D., and **Weaver, V.M.** Characterizing Integrin Dependent Force Transduction. BMES Annual Meeting, Philadelphia, PA, October 2004.

3. Lakins, J.N. and **Weaver, V.M.** RNA interference of PTPN12 enhances acinar growth of MCF10A cells potentially through activation of Rho. Poster Presentation. 44th American Society of Cell Biology Annual Meeting, Washington, D.C. December 2004.

4. Paszek, M., Zahir, N., Lakins, J.N., Johnson, K.R., Rozenberg, G.I., Dembo, M., Boettiger, D., Hammer, D., and **Weaver, V.M.** Mechano-signaling in mammary morphogenesis and tumorigenesis Poster Presentation. 44th American Society of Cell Biology Annual Meeting, Washington, D.C. December 2004.

5. **Weaver VM,** Johnson KR, Lakins JN. Adhesion-linked protein tyrosine phosphatases, morphogenesis, and breast cancer progression. Poster Presentation. Era of Hope Meeting for the Department of Defense (DOD) Breast Cancer Research Program (BCRP), Philadelphia, PA, June 2005.

C. Oral Meetings Presentations:

1. **Weaver, V.M.** Outside in Signaling: The importance of the Breast Tissue Microenvironment. Third Annual Future of Breast Cancer: An International Congress, "New concepts in Eradicating Breast Cancer - Novel and Established Growth, Survival Mechanisms, and Clinical Implications", Hamilton, Bermuda, July 24, 2004.

2. **Weaver, V.M.** Tissue tension: mechanics meets mammary morphogenesis and malignancy. 2004 VCC Research Symposium: "The Course of Cancer" October 7, Burlington, Vermont, 2004.

3. Paszek MJ, Zahir N, Lakins JN, Johnson KR, Rozenberg G, Dembo M, Hammer DA, **Weaver VM.** Mechano-signaling in Mammary Morphogenesis and Tumorigenesis. Platform Presentation. ASCB 44th Annual Meeting, Washington, DC, 2004.

4. **Weaver, V.M.** Form, fate and matrix flexibility. Special Workshop on Senescent Cells and their Environment, Novato, CA, January 24, 2005.

5. **Weaver, V.M.** Tensional force, cell signaling and epithelial differentiation and survival. Gordon Conference on Radiation Oncology, Ventura, CA, February 2, 2005.

6. **Weaver, V.M.** Tissue polarity and the mechanism of epithelial resistance to chemotherapy, Keystone Meeting on Microenvironment in Tumor Induction, Banff, Alberta, Canada, February 7, 2005.

7. **Weaver, V.M.** Tensional regulation of tissue morphogenesis and malignant transformation, Lorne Cancer Conference, Melbourne, Australia, February 12, 2005.

8. **Weaver, V.M.** Tumor microenvironment in the 3rd Dimension, AACR Major Symposium on Stromal-epithelial interactions, Anaheim, CA, April 18, 2005.

9. **Weaver, V.M.**Microenvironmental forces driving malignant transformation and metastasis, Joint IRCC and EMBO Alpine Spring Conference on 'The invasive growth program: Concepts and technologies, Torino, Italy, May 27, 2005.

10. **Weaver, V.M.** Spatial-mechanical regulation of life and death of a mammary epithelium, 38th Annual Meeting of the Japanese Society of Developmental Biology, Sendai, Japan, June 3, 2005.

11. **Weaver, V.M.**Tissue architecture and Pathology, Era of Hope DOD Meeting, Philadelphia, PA, June 11, 2005.

12. **Weaver, V.M.** Force-dependent spatial regulation of signaling and morphogenesis, Gordon Conference on Mechanisms of Cell Signaling, Hong Kong University of Science and Technology, Hong Kong, China, June 14, 2005.

13. **Weaver, V.M.** Implications of matrix remodeling on tumor progression, NCI special meeting on Mouse Models of Human Cancers Consortium, New Brunswick, New Jersey, June 29, 2005.

14. **Weaver, V.M.** Spatial-Mechanical regulation of mammary morphogenesis and malignancy, ASCB-ECI Conference on Engineering Cell Biology, Washington, Seattle, July 16, 2005.

D. Invited Institutional Presentations:

1. **Weaver, V.M.** Stromal-epithelial iteractions and tension-dependent breast transformation, Lombardi Comprehensive Cancer Center, Georgetown, VA. September 10, 2004

2. **Weaver, V.M.** Quantitative approaches to mammary gland biology and neoplasia, Miami School of Medicine, Miami, Florida, October 19, 2004.

3. **Weaver, V.M.** The tension mounts: Mechanics meets mammary morphogenesis and malignancy, MIT, Department of Bioengineering, Boston, MA, January 14, 2005.

4. **Weaver, V.M.** The tension mounts: mechanisms meets mammary morphogenesis and malignancy, Department of Cell Biology, UMass Medical School, Worcester, MA, March 23, 2005.

5. **Weaver, V.M.**Tension, Tumors and Trauma, NIH NIDCR, Bethesda, MA, April 7, 2005.

6. **Weaver, V.M.**Form, fate and flexibility, Vontz Center, University of Cincinnati College of Medicine, Cincinnati, OH, May 12, 2005.

7. **Weaver, V.M.** Tensional-homeostasis and morphogenesis, Center for Developmental Biology, Kobe, Japan, June 7, 2005.

Conclusions:

Since our last progress report we completed the confirmation and analysis of expression profiles of PTPMEG1 and D1 (as well as an array of other PTPs) in nonmalignant, malignant and phenotypically transformed human MECs both in 2D and in 3D rBM culture formats. This has been achieved at the RNA and protein levels – although the protein analysis studies still need to be reconfirmed using an improved antibody that we are in the process of generating. In this respect we have procured an antibody specific to PTPMEG1 and have successfully used this for immunoblotting purposes. However, the antibody is not as specific as is required to be used for reliable IP and substrate identification studies. Furthermore, we have conducted a number of immunostaining studies using this antibody to examine the localization of endogenous PTPMEG1 in nonmalignant and transformed MECs in both 2D and 3D culture and during various stages of mammary morphogenesis. While we could obtain immunostaining images, due to the relatively poor quality of the antibody we were unsatisfied with our results and felt that we could not draw definitive conclusions regarding PTPMEG1 localization in mammary tissue in rBM. For these reasons we have attempted two different but complimentary strategies which we believe will aid in our studies. We have generated various directly tagged PTPMEG1 expression constructs – including an EGFP direct tagged PTPMEG1 and a triple HA-tagged construct. For various reasons the EGFP-tagged expression constructs do not appear to be well-tolerated in MECs, hence we are in the process of conducting experiments using the HA-tagged construct in nonmalignant MECs and will report these results in our next years progress report. As outlined in our work aims we have also decided to generate polyclonal and monoclonal antibodies that we hope to use to pull down/substrate identification studies as well as for immunostaining purposes and we are currently in the process of generating purified recombinant proteins for this purpose.

Interestingly, when we assessed the effect of phenotypic reversion on PTPMEG1 and D1 expression we noted that PTPMEG1 levels increased significantly and remained quite elevated in the reverted tumor structures while PTPD1 levels declined substantially. Although we earlier reported that phenotypic reversion did not appear to modulate PTP expression – those conclusions were based upon preliminary studies. We are basing our current data upon an exhaustive analysis of PTP expression in multiple experiments. These data suggest that PTPMEG1 may be required to maintain cell-cell adhesions – but also that mechanisms that exist in nontransformed MECs to regulate PTPMEG1 expression may not exist in tumors. Again these observations indicate that we will need to identify putative PTPMEG1 specific substrates to understand PTPMEG1 effects on tissue behavior.

Finally, this past year we have begun in earnest to study the role of ECM stiffness on tissue behavior. We have found that at least part of the explanation for the dramatic differences between MEC behavior in vivo and in rBM as compared to conventional tissue culture plastic resides in the fact that

tissue culture plastic is substantially and significantly stiffer. This matrix stiffness has profound effect on tissue morphogenesis, especially cell-cell junctional integrity and polarity and mediates these effects via regulation of integrin focal adhesions. We also determined that tumors with elevated focal adhesion and heightened cell contractility phenotypes can be normalized phenotypically be inhibiting myosin dependent force generation. Finally, we found that PTPMEG1 expression is tightly regulated by integrin adhesions – but more specifically is dependent for integrin-mediated force. Thus, PTPMEG1 may be abnormally induced in tumors because transformed tissues are stiffer and consequently elevate integrin adhesion maturation – a possibility that we intend to examine in more detail over the next year (see Figures 4-6).

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Appendix I

PTP (Locuslink)	Synonyms (From Locuslink)	Amplified PTP Domain (N or C Terminal)	Molecular Wt (nts) of Expected RFDD Band with 5' or 3' Primer End Labeled								Intensity of RFDD Band
			AluI (3')	AluI (5')	DpnII (3')	DpnII (5')	MspI (3')	MspI (5')	RsaI (3')	RsaI (5')	
PTPN12	PTP-PEST, PTPG1	N/A	72 (Y)	296 (Y)	329 (Y)	35 (Y)	None	None	None	None	(++)
PTPN13	FAP1, PTP1E, PTP-BAS, PTPL1	N/A	206 (Y)	165 (Y)	42 (Y)	285 (Y)	None	None	None	None	(++)
PTPN21	PTPD1, PTPRL10	N/A	324 (Y)	83 (Y)	55 (Y)	143 (Y)	190 (Y)	153 (Y)	316 (Y)	91 (Y)	(++)
PTPN1	PTP1B, PTP-1B	N/A	194 (Y)	192 (Y)	152 (Y)	119 (Y)	47 (Y)	337 (Y)	None	None	(++)
PTPN6	HCP, HCPH, SHP-1, PTP-1C, SHP-1L	N/A	87 (Y)	248 (Y)	71 (Y)	224 (Y)	None	72 (Y)	139 (Y)	180 (Y)	(++)
PTPN9	MEG2	N/A	133 (Y)	248 (Y)	293 (Y)	116 (Y)	None	None	322 (Y)	91 (Y)	(++)
PTPN4	PTPMEG	N/A	None	None	137 (Y)	230 (Y)	None	None	65 (Y)	238 (Y)	(++)
PTPN2	PTPT, Tcell PTP	N/A	100 (Y)	147 (Y)	*26*	*353*	None	None	201 (Y)	91 (Y)	(+)
PTPRU	PI, FMI, PTP, PCP-2, PTP-J, PTPRO, PTPU2, GLEPP1, HPTP-J, PTP-PI, R-PTP-PSI	N (Isoform 1)	308 (Y)	57 (Y)	314 (Y)	47 (Y)	40 (Y)	97 (Y)	273 (Y)	91 (Y)	(++)
		N (Isoform 2)	299 (Y)	57 (Y)	305 (Y)	47 (Y)	40 (Y)	88 (Y)	273 (Y)	82 (Y)	(++)
		C	226	57	None	None	117	111	289	94	
PTPRA	LRP, HLPR, PTPA, HPTPA, RPTPA, PTPRL2 (alpha)	N	126	254	92	284	None	None	207	91	
		C	207 (Y)	164 (Y)	86 (Y)	218 (Y)	147(Y)	213 (Y)	280 (Y)	91 (Y)	(+)
PTPRK	R-PTP-kappa	N	None	None	None	None	72 (Y)	72 (Y)	*27*	199 (Y)	(++)
		C	None	None	108	161	36	168	145	33	
PTPRG	PTPG, HPTPG, RPTPG, D3S1249 (gamma)	N	58 (Y)	343 (Y)	66 (Y)	84 (Y)	None	None	86 (Y)	314 (Y)	(+)
		C	162	65	331	35	None	None	82	88	
PTPRS	(sigma)	N	212	156	*360*	74	41	247	None	None	
		C	317 (Y)	57 (Y)	*355*	35 (Y)	125 (Y)	72 (Y)	250 (Y)	91 (Y)	(+)
PTPRF	LAR	N	149 (Y)	156 (Y)	280 (Y)	84 (Y)	294(Y)	72 (Y)	100 (Y)	91 (Y)	(+)
		C	317	57	None	None	150	198	*359*	91	
PTPRJ	DEP1	N/A	None	None	None	None	81 (Y)	160 (Y)	79 (Y)	295 (Y)	(+/-)